

Genetic Variability in Two Brazilian Ethnic Groups: A Comparison of Mitochondrial and Protein Data

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ABSTRACT Sequence data from the first hypervariable segment of the mitochondrial DNA control region of 124 subjects belonging to three African-Brazilian and three Brazilian Indian populations were compared with information related to 12 protein genetic loci from 601 persons living in the same localities. There is high diversity among the mtDNA sites, and the most variable in one ethnic group are not the most variable in the other. No differences in gene diversity between populations within ethnic groups were observed, but the Indians showed a reduced variability. Much more interpopulation variation was observed in the mtDNA data than in the protein set. The relationships obtained for the six populations, however, are the same regardless whether mtDNA or protein loci are considered. African-Brazilians from Porto Alegre and Salvador, situated 3,000 km apart, are more similar to each other than both are to Paredão, despite the geographical proximity between Porto Alegre and Paredão, which are just 50 km apart. The tree topology in relation to the three Indians groups, on the other hand, is that expected when languages, culture, and geography are considered. *Am J Phys Anthropol* 103:147-156, 1997. © 1997 Wiley-Liss, Inc.

Studies of human populations based on blood and protein polymorphisms have revealed much about our variability (Nei and Roychoudhury, 1982, 1993; Salzano and Callegari-Jacques, 1988; Cavalli-Sforza et al., 1988, 1994). However, our knowledge of population genetic diversity, over the last decade, has improved considerably with the application of molecular techniques. The delineation of mitochondrial DNA (mtDNA) variation has provided unique and often startling new insights into human evolution (Cann et al., 1987; Vigilant et al., 1991; Stoneking et al., 1992; Horai et al., 1993, 1995; Torroni et al., 1992, 1993, 1994a, 1994b; Szathmary, 1993; Torroni and Wal-

lace, 1995; Wallace, 1995; Chen et al., 1995; Bonatto et al., 1996; Easton et al., 1996; Foster et al., 1996; Merriwether et al., 1991, 1996; Monsalve et al., 1996a, 1996b; Ribeiros-Santos et al., 1996; Santos et al., 1996; Ward et al., 1991, 1996; Bonatto and Salzano, 1997). Some of these investigations

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have shown that Africans are the most variable of the ethnic groups, supporting suggestions that the last common ancestor of contemporary humans existed some 100,000–200,000 years ago in Africa, although other interpretations have been put forward (Excoffier and Langaney, 1989; Templeton, 1992, 1993). Others have revealed that Amerindians show low genetic variability when they are compared with other human groups, and that this may be due to the depopulation effect started by the European colonization in the 16th century. The suggestion that the colonization from Asia into the Americas was accompanied by severe bottlenecks that markedly restricted the levels of diversity of these populations was also challenged (Baillet et al., 1994; Easton et al., 1996). The degree of agreement between results obtained from mtDNA and protein data, however, has rarely been examined (Bowcock et al., 1994; Nei and Takezaki, 1996), and no study has compared both types of genetic variation in the same sample. Further, few investigations have considered the partition of the total gene diversity in its intra- and interpopulational diversity (Livshits and Nei, 1990; Bortolini et al., 1995; Deka et al., 1995; Bortolini and Salzano, 1996; Urbanek et al., 1996; Zago et al., 1996).

Historically, the Brazilian coast was reached on 22 April 1500, six weeks after the fleet commanded by Pedro Alvares Cabral left Lisbon. On the next day, the region around the place of anchorage was explored and the first contacts were established with inhabitants of the land who, following the Colombian tradition, were called Indians. At that time Brazil had about five million inhabitants; Ribeiro, 1996. Recently, genetic studies of extant Brazilian Indians have been performed at the protein (Salzano and Callegari-Jacques, 1988; Salzano et al., 1991; Callegari-Jacques et al., 1994, 1996; Cavalli-Sforza et al., 1994; Salzano et al., 1997), nuclear DNA (Guerreiro et al., 1992; Petzl-Erler and McDevitt, 1994; Bevilacqua et al., 1995; Heidrich et al., 1995; Pena et al., 1995; Santos et al., 1996; Zago et al., 1996; Hutz et al., 1997), and mitochondrial DNA (Bonatto et al., 1996; Santos et al., 1996; Ward et al., 1996; Bonatto and Salzano, 1997) levels. The first Africans were introduced in Brazil

about three decades after the arrival of European colonizers, and during the Colonial slave trade period about six million of them were forced to migrate to this country (Ribeiro, 1996). Present-day African-Brazilians are now the product of an original African gene pool that received European and Indian genes for about 12 generations. These populations have been studied at the protein (Azevêdo, 1980; Azevêdo et al., 1980; Weimer et al., 1981; Franco et al., 1982, 1986; Schneider et al., 1987; Bortolini et al., 1992, 1994, 1995, 1997a) and more recently at the nuclear DNA (Zago et al., 1992; Silva and Figueiredo, 1994; Heidrich et al., 1995) and mtDNA (Bortolini et al., 1997b) levels.

In the present study we have estimated the degree of mtDNA single-site nucleotide diversity at the first hypervariable segment of its control region and the corresponding level of intra- and interpopulation gene diversity in six Brazilian populations (three African-Brazilian and three Brazilian Indian groups). We also compared the levels of genetic variability calculated from the mtDNA and classical polymorphism data sets, considering the relationships observed among the six populations with the two kinds of results.

SUBJECTS AND METHODS

The African-Brazilian sample consists, for the mtDNA sequence studies (360 nucleotides of the first hypervariable segment, HVS-I), of 42 individuals from three populations: 1) Porto Alegre (POA; 30°5'S, 51°10'W; the capital of Brazil's southernmost state, Rio Grande do Sul); 2) Salvador (SAL; 12°55'S, 38°29'W; capital of the Brazilian northeastern state of Bahia); and 3) Paredão (PAR; 28°20'S, 50°90'W; a rural group living in the state of Rio Grande do Sul, with about 100 inhabitants and which assembled in the last century, probably composed by fugitive slaves). These results have been presented in detail by Bortolini et al. (1997b) and the protein data, which included 12 loci and larger sample sizes (109 each for Porto Alegre and Salvador and 36 for Paredão), by Bortolini et al. (1992, 1995, 1997a).

The Brazilian Indian populations are represented by three Amazonian tribes: 1) Xavante (XAV), who speak a Ge language, of

the Macro-Ge subdivision of the Ge-Pano-Carib group (Rodrigues, 1986; Greenberg, 1987), and whose village, from which the material was collected, is situated in the State of Mato Grosso (51°40'W, 13°20'S); 2) Zoró (ZOR), whose language is classified in the Tupi stock, Mondé family (Rodrigues, 1986), and who live in a single village, also located in Mato Grosso (60°20'W, 10°20'S); and 3) Gavião (GAV), also a Tupi-Mondé group, living in the State of Rondônia (61°8'W, 10°10'S). They should not be confounded with Ge-speaking groups with the same name living in the States of Pará or Maranhão. The mtDNA of 82 individuals has been sequenced for the HVS-1, and the results were reported by Ward et al. (1996). The protein data for these same Indian populations, which include 23 loci and larger sample sizes (78 for Zoró, 183 for Gavião and 86 for Xavante), have been generated in our laboratory and are still unpublished. For this study, we have only considered the protein loci which were studied in both the African-Brazilian and Brazilian Indian populations. They were acid phosphatase (ACP), adenylate kinase (AK), esterase D (ESD), glyoxalase I (GLO), glucose-6-phosphate dehydrogenase (G6PD), phosphoglucosmutase 1 (PGM1), phosphogluconate dehydrogenase (PGD), hemoglobin (HB), haptoglobin (HP), transferrin (TF), albumin (AL), and ceruloplasmin (CP).

The frequency of variants in each variable site, in each population, was compared to the reference sequence (Anderson et al., 1981), and the differences inferred from direct sequence comparisons. The single-site nucleotide diversity estimates and the partition of these into intra- and interpopulational components were performed using Nei's statistics (*Ht*, *Hs* and *Gst'*; Nei, 1973, 1986, 1987) and the DISPAN computer program (Ota, 1993). Gene diversity for the mtDNA data was estimated according to two methods. The first method was from a matrix of p-distances. The latter are calculated as the proportion (p) of nucleotide sites at which the two sequences compared are different. This is obtained by dividing the number of nucleotide differences by the total number of nucleotides compared. They were obtained using the MEGA program (Kumar et

al., 1993). Afterward, the intra- and interpopulational diversity were evaluated according to Nei (1987) and Nei and Jin (1989) using a computer program kindly made available to us by Lynn B. Jorde (University of Utah). The standard errors of the estimates were obtained with the SEND program (Jin, 1989). The second method was from the nucleotide frequencies in each variable site, using Nei's statistics (1973, 1986, 1987) and the DISPAN program. The diversity values from the protein data set were obtained from the allele frequencies, also using Nei's statistics and the DISPAN computer program.

Genetic distances between populations and their relationships, using both data sets, were calculated by two methods: 1) the D_A distance (Nei et al., 1983; Nei and Roychoudhury, 1993) and 2) the standard genetic distance (D_S ; Nei, 1987). The relationships among populations were then evaluated from a neighbor-joining (NJ) tree (Saitou and Nei, 1987), which was statistically evaluated by the bootstrap test (Felsenstein, 1985) with 2,000 replications (Hedges, 1992), using DISPAN.

RESULTS

Table 1 summarizes the information on 60 variant sites (between positions 16024 and 16380 of the mtDNA control region), relative to the Anderson et al.'s (1981) sequence, observed in the six investigated populations. As expected, transitions (92%) were more prevalent than transversions. Three sites (16114, 16286, and 16293) differed by both transitions and transversions and four (16188, 16248, 16265, and 16358) by transversions only.

Table 1 also shows the diversity analysis for each variable site. The total variability (*Ht*), considering African-Brazilians, ranges from 0.024 (in sites 16080, 16111, 1650, 16173, 16192, 16217, 16248, 16260, 16264, 16270, 16292, 16295, 16316, 16354, and 16355) to 0.478 (site 16223), the corresponding values among the Indians being from 0.022 (sites 16256, 16266, 16316) to 0.5 (16189 and 16325). The partition of the total single-site nucleotide diversity into its intra- and interpopulational components reveals that, for African-Brazilians, site 16172

TABLE 1. Nucleotide variant frequencies ($\times 100$) and single-site diversity analyses for the HVS-I of the mtDNA control region of subjects from six Brazilian populations¹

Nucleotide position ²	Population			Single-site diversity			Populations			Single-site diversity		
	POA	SAL	PAR	Total Ht	Intrapop. Hs	Interpop. Gst' (%)	XAV	ZOR	GAV	Total Ht	Intrapop. Hs	Interpop. Gst' (%)
16051A	G (3.6)	—	G (40.0)	0.248	0.183	34.8	—	—	—	—	—	—
16080A	G (3.6)	—	—	0.024	0.023	3.6	—	—	—	—	—	—
16092T	—	—	—	—	—	—	—	—	—	—	—	—
16093T	C (7.2)	—	—	0.047	0.044	—	C (36.0)	—	C (3.7)	0.024	0.023	3.7
16111C	T (3.6)	—	—	0.024	0.023	3.6	T (16.0)	—	—	0.273	0.229	22.5
16114C	A/T (7.2/3.6)	—	—	0.070	0.066	8.6	—	—	T (14.8)	0.281	0.280	0.5
16124T	C (7.2)	—	—	0.047	0.044	7.2	—	—	—	—	—	—
16126T	C (3.6)	—	—	0.093	0.089	6.7	—	—	—	—	—	—
16129G	A (28.8)	C (11.1)	—	0.231	0.202	17.2	—	—	—	—	—	—
16148C	T (14.4)	A (11.1)	—	0.155	0.148	7.1	—	—	—	—	—	—
16150C	T (3.6)	T (11.1)	—	0.024	0.023	3.6	—	—	—	—	—	—
16168C	T (10.3)	—	—	0.066	0.062	10.3	—	—	—	0.026	0.025	3.3
16172T	C (36.0)	C (22.2)	C (60.0)	0.477	0.429	14.6	T (4.0)	—	—	—	—	—
16173C	T (3.6)	—	—	0.024	0.023	3.6	—	—	—	—	—	—
16175A	—	—	—	—	—	—	—	G (6.7)	G (22.2)	0.174	0.157	14.2
16187C	T (36.0)	—	—	0.211	0.154	36.0	—	—	—	—	—	—
16188C	G (14.4)	—	—	0.091	0.082	14.0	—	—	—	—	—	—
16189T	C (57.6)	C (22.2)	—	0.390	0.278	37.8	C (84.0)	—	C (16.6)	0.500	0.348	39.5
16192C	T (3.6)	—	—	0.024	0.023	3.6	—	—	—	—	—	—
16193C	—	—	T (20.0)	0.124	0.107	20.0	—	—	—	—	—	—
16209T	C (7.2)	C (11.1)	—	0.114	0.110	5.5	—	—	—	—	—	—
16213G	A (3.6)	—	A (20.0)	0.145	0.130	14.9	—	—	—	—	—	—
16217T	C (3.6)	—	—	0.024	0.023	3.6	C (84.0)	C (3.3)	C (14.8)	0.499	0.195	66.1
16223C	T (93.6)	T (77.7)	T (100.0)	0.478	0.215	64.7	T (16.0)	T (96.4)	T (85.2)	0.499	0.195	66.0
16230A	G (10.8)	—	—	0.069	0.064	10.8	—	—	—	—	—	—
16239C	—	—	T (20.0)	0.124	0.106	20.0	—	—	—	—	—	—
16241A	—	—	—	—	—	—	G (36.0)	—	—	0.211	0.154	36.0
16248C	A (3.6)	—	—	0.024	0.023	3.6	—	—	—	—	—	—
16256C	—	—	—	—	—	—	—	T (3.3)	—	0.022	0.021	3.3
16260C	T (3.6)	—	—	0.024	0.023	3.6	—	—	—	—	—	—
16261C	—	—	—	—	—	—	—	—	T (14.8)	0.094	0.084	10.4
16264C	T (3.6)	—	—	0.024	0.023	3.6	—	—	—	—	—	—
16265A	C/T (10.3/3.6)	—	T (20.0)	0.078	0.082	11.2	—	—	—	—	—	—
16266C	—	—	—	—	—	—	—	T (3.3)	—	0.022	0.021	3.3
16270C	T (3.6)	—	—	0.024	0.023	3.6	—	—	—	—	—	—
16278C	T (36.0)	T (22.0)	T (20.0)	0.385	0.375	3.8	—	—	—	—	—	—
16284A	—	—	—	—	—	—	—	T (20.0)	T (11.1)	0.186	0.172	10.4
16286C	A/G (3.6/7.2)	T (11.1)	—	0.139	0.132	7.2	—	—	G (20.0)	0.124	0.107	20.0
16290C	—	T (11.1)	—	0.071	0.066	11.1	—	—	—	—	—	—
16291C	—	—	—	—	—	—	T (16.0)	T (20.0)	T (14.8)	0.281	0.280	0.5
16292C	T (3.6)	—	—	0.024	0.023	3.6	—	—	T (11.1)	0.128	0.109	20.7
16293A	G/T (10.8/3.6)	G (11.1)	—	0.157	0.150	6.3	—	—	—	—	—	—
16294C	T (18.0)	T (22.0)	T (20.0)	0.321	0.320	0.2	—	—	—	—	—	—

[illegible]

[†] Dashes indicate the same nucleotide of the reference sequence and no diversity. Numbers in parentheses are the variant frequencies in percentages.

² According to Anderson et al. (1981).

POA, Porto Alegre; SAL, Salvador; PAR, Paredão; XAV, Xavante; ZOR, Zoró; GAV, Gavião.

Table 2 presents further gene diversity information. Three different indices (two generated from the mtDNA data and one from the protein results) were calculated. The first index expresses gene diversity considering the average of the nucleotide differences between sequences in the population. The values range from 0.008 (XAV) to 0.024 (POA), reflecting the proportion of variable sites in the HVS-I, which are of 3% and 12%, respectively. The second summarizes the variation considering the frequencies of variant nucleotides, given in Table 1. Once more the Xavante showed the lowest value (0.049) and Porto Alegre the highest number (0.136).

The third index evaluates the variability based on the allele frequencies in 12 polymorphic protein loci. The Gavião sample showed the lowest number (0.155), and Salvador sample the highest (0.214). All the values observed for the African-Brazilians lie within the 0.199–0.231 range seen in Africans (Livshits and Nei, 1990; Nei and Takezaki, 1996); the same is true for the Brazilian Indian numbers and those found in other South Amerindians, 0.123–0.167 (Livshits and Nei, 1990; Salzano et al., 1997).

When the three samples in each of the two ethnic groups, respectively, are considered as a whole Amerindians display lower values, but the standard errors of the estimates show that these values are not significantly different.

Table 2 also gives the portion of total gene diversity attributable to the interpopulational variability (G_{st}). In the mtDNA data set, African-Brazilians show less interpopulation differentiation (15%/19%) than the Brazilian Indians (34%/36%). The protein data indicate less interpopulation variation, 3%, for both ethnic groups.

Figure 1 shows the NJ tree based on the mtDNA data set using a D_A distance matrix. It has the same topology as those of all the

TABLE 2. Gene diversity in six Brazilian populations considering mitochondrial and protein data

Population	mtDNA				Protein		
	No. of individuals	Variable sites (%)	Gene diversity \pm SE		No. of individuals	No. of loci	Gene diversity \pm SE ³
			A ¹	B ²			
African-Brazilians			<i>Gst'</i> (15%) ⁴	<i>Gst'</i> (19%) ⁴			<i>Gst'</i> (3%) ⁴
Porto Alegre (POA)	28	12	0.024 \pm 0.003	0.136 \pm 0.019	109	12	0.205 \pm 0.054
Salvador (SAL)	9	5	0.014 \pm 0.003	0.082 \pm 0.016	109	12	0.214 \pm 0.057
Paredão (PAR)	5	4	0.012 \pm 0.004	0.074 \pm 0.020	36	12	0.208 \pm 0.066
Total	42	14	0.019 \pm 0.003	0.107 \pm 0.018	254	12	0.212 \pm 0.059
Brazilian Indians			<i>Gst'</i> (34%) ⁴	<i>Gst'</i> (36%) ⁴			<i>Gst'</i> (3%) ⁴
Xavante (XAV)	25	3	0.008 \pm 0.003	0.049 \pm 0.015	86	12	0.159 \pm 0.059
Zoró (ZOR)	30	5	0.011 \pm 0.003	0.064 \pm 0.015	78	12	0.162 \pm 0.063
Gavião (GAV)	27	4	0.012 \pm 0.003	0.068 \pm 0.017	183	12	0.155 \pm 0.058
Total	82	6	0.014 \pm 0.003	0.080 \pm 0.016	347	12	0.161 \pm 0.060

¹ Using formulae 2 and 3 given in Nei and Jin (1989).

² Using formulae 8.6 and 8.14 given in Nei (1987), considering the variant nucleotide frequencies given in Table 1.

³ Using formulae 8.6 and 8.14 given in Nei (1987), considering the allele frequencies. The numbers of the Amerindians were calculated from unpublished data of our laboratory.

⁴ Since *Gst* (Nei, 1973) is known to be affected by the number of subpopulations examined, *Gst'* (Nei, 1986) was calculated.

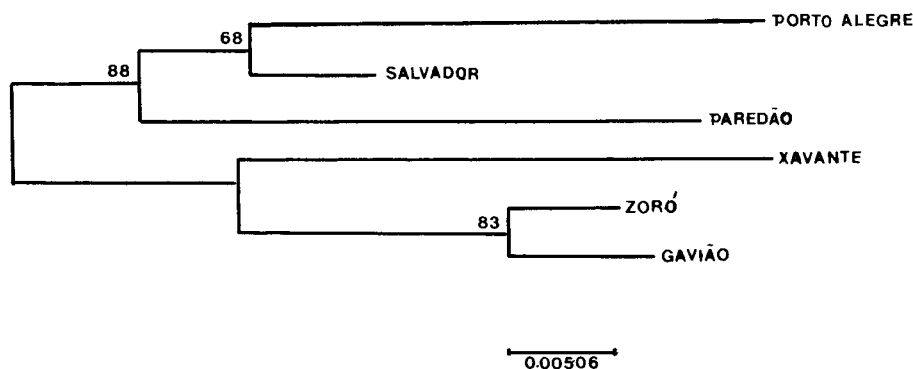


Fig. 1. Neighbor-joining tree generated from a D_A distance matrix, employing the HVS-I mtDNA data. Bootstrap values, indicating the degree of support for each branch point, are shown as the percent of 2,000 replicates consistent with each branch point. The tree was rooted using the midpoint rooting.

other trees we have constructed, including those generated from the protein data set (not shown). There is a clear split separating African-Brazilians from the Indian populations, which is consistent in 88% of the bootstraps. The African-Brazilian cluster indicates that the individuals from Porto Alegre are more closely related to those from Salvador than to those from Paredão, despite the fact that the latter live much closer (about 50 km) from Porto Alegre. In the Indian cluster the Xavante show a clear distinction from the other two tribes, which group together in 83% of the bootstraps.

DISCUSSION

The points to be emphasized in our results are: 1) no differences in gene diversity are

observed within the ethnic groups considered, but there is a trend toward reduction in mtDNA diversity among the Indians, and the protein results point in the same direction; 2) there is also much more interpopulation variability in the mtDNA data than in the protein set; and 3) the single-site nucleotide diversity analysis reveals high heterogeneity within the HVS-I region and between ethnic groups. Several studies have shown that the number of substitutions per site does not follow a Poisson distribution, expected under a homogeneous model of change in the mitochondrial control region (Hasegawa et al., 1993; Wakeley, 1993). A few sites are the target of most mutation events, and act as mutational hot spots, while the majority are not variable (Aris-Brosou and

Excoffier, 1996). Other factors may influence the interethnic differences. The total gene diversity observed in African-Brazilians, in both data sets, reveals similarity with those obtained from Africans. Since just a subsample of Africans was brought to Brazil as slaves (Africa had about 100 million inhabitants at slavery time; Curtin et al., 1991), the high level of variability in the Brazilian populations may be due to admixture. There are several suggestions that admixed populations may show higher gene variability when compared with that of the source populations from which they were derived (Byard et al., 1985), although this notion is not universally valid (Chakraborty, 1986).

No previous *Gst'* estimates for mtDNA sequences considering human populations have been performed to date, and only one study has used the *Gst* statistic, which however was not corrected considering the number of subpopulations (20% for major continental groups; Jorde et al., 1995). Estimates of *Gst'*, using population frequencies of mtDNA haplotypes and haplogroups in a large number of Native American populations, have furnished results which are similar to those observed here (30% and 31%; Bortolini and Salzano, 1996). The mtDNA differentiation between the three Indian tribes studied here is higher than those for micro- and minisatellite loci, calculated from other Amerindian sets (*Gst'* = 11%–13%; Deka et al., 1995; Urbanek et al., 1996; Zago et al., 1996). It is also higher than that obtained for African-Brazilians, and this could reflect either structural differences between the two sets of populations or more variance in the Indians' female contribution. The level of interpopulational diversity, considering classical polymorphisms, is uniformly low among African-Brazilians and Brazilian Indians (3%), in agreement with earlier studies with other population sets (1.3%–6%; Livshits and Nei, 1990; Bortolini et al., 1995, 1997a).

The substantially higher mtDNA *Gst'* values may be attributed to the fact that the effective population size of this organelle is one-fourth that of nuclear DNA (Birky et al., 1983), leading to a higher degree of genetic drift. In fact, this particular feature of the mitochondrial genome, rather than its higher

mutation rate, is probably mainly responsible for its high level of population differentiation. Previous findings have shown that high mutation rates produce low *Gst/Gst'* values due to a high level of within-group diversity relative to total variability (Chakraborty and Jin, 1992; Jin and Chakraborty, 1995; Deka et al., 1995; Jorde et al., 1995).

The tree topology suggests genetic affinity between the African-Brazilians from Porto Alegre and Salvador, situated 3,000 km apart. This relationship has been evaluated by Bortolini et al. (1997a), who suggested that this similarity could be due to the sharing of similar European genetic stocks, rather than African stocks. Historical documents and genetic studies indicate that the origins of the Africans who colonized the South/Southeast and Northeast of Brazil are different (Goulart, 1975; Cardoso, 1977; Maestri-Filho, 1984; Zago and Figueiredo, 1993). On the other hand, the amount of European contribution to these two populations is substantial (38% for Salvador; 59% for Porto Alegre; Bortolini et al., 1995, 1997a) and in both cases the main contributors have been Portuguese. Paredão is a rural, small, semi-isolated population, and its difference from Porto Alegre could be due to random genetic drift or founder effects. The genetic similarity among the Zoró and Gavião Indians is expected, since they speak similar languages, have many cultural traits in common, and live in relatively close geographical proximity (Ward et al., 1996).

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